Litton BIONETICS

MUTAGENICITY EVALUATION OF FDA 71-12 GUM TRAGACANTH

Final ~~DRAFT~~ REPORT

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MUTAGENICITY EVALUATION

OF

FDA 71-12 GUM TRAGACANTH

Final

[redacted]T REPORT

SUBMITTED TO

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Litton BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 71-12 Gum Tragacanth, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: December, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 71-12 Gum Tragacanth

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: December 29, 1977

2. Description: Beige powder

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: *Saccharomyces cerevisiae*, strain D4

Bacteria Strains: *Salmonella typhimurium*, strains TA-1535
TA-1537
TA-1538
TA-98
TA-100

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse - ICR random bred adult males; rat - Sprague-Dawley adult males; and monkey - Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1

POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
Nonactivation	Methylnitrosoguanidine	Water or saline	BPS[b]
	Ethylmethanesulfonate	Water or saline	BPS[b]
	2-Nitrofluorene	Dimethylsulfoxide[c]	FS[b]
	Quinacrine mustard	Water or saline	FS[b]
Activation	Dimethylnitrosamine	Water or saline	BPS[b]
	2-Acetylaminofluorene	Dimethylsulfoxide[c]	FS[b]
	8-Aminoquinoline	Dimethylsulfoxide[c]	FS[b]
	2-Aminoanthracene	Dimethylsulfoxide[c]	BPS[b]

[a] Concentrations given in the Results Section
[b] BPS = base-pair substitution; FS = frameshift
[c] Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for the test chemical were determined prior to screening.

The test chemical was tested for toxicity against specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival concentrations and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for the chemical with a given strain, then a maximum dose of 5% (w/v) was used.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic, 24-well tissue culture plates (Linbro). Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C. The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the nonactivation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for nonactivation tests.

D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. The data were then processed and printed from a computer program. All raw data sheets are dated and signed by the responsible technician.

IV. RESULTS SECTION

A. Solubility Properties of the Test Compound:

1. Name or code designation of the test compound: FDA 71-12 Gum Tragacanth

2. Test solvent: *Acetone**

3. Solubility of the test compound under treatment conditions: Insoluble (Suspension of compound)

4. Additional comments: Beige powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: 7/11/77

2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	1.1	1.25
1/2 50% Survival	2.2	2.50
50% Survival	4.4	5.0

*The concentration of solvent was equal to the highest volume of test material added.

**Could not be used as solvent control at .25 ml per well (highest volume of test compound added) because of toxicity to bacterial cells. Only .1ml per well was used.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000000004
B. TEST DATE: AUG. 11, 1977

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE									
				TA-1535		TA-1537		TA-1538		TA-98		TA-100	
				1	2	1	2	1	2	1	2	1	2
1. NONACTIVATION													
SOLVENT CONTROL*		---	---	15	24	18	12	11	19	25	25	132	137
POSITIVE CONTROL**		---	---	899	>1000	506	478	590	782	839	573	928	778
TEST	4.40000 %	---	---	23	18	11	12	19	15	31	37	137	139
	2.20000 %	---	---	17	20	18	13	17	18	29	25	144	149
	1.10000 %	---	---	15	18	19	17	21	16	38	34	132	145
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	24	23	19	15	15	19	39	47	158	188
		RAT	LIVER	25	25	38	32	19	31	33	50	160	167
		MONKEY	LIVER	12	14	14	15	17	12	47	38	231	203
POSITIVE CONTROL***		MOUSE	LIVER	231	307	184	192	>1000	>1000	>1000	>1000	484	490
		RAT	LIVER	298	344	276	239	>1000	>1000	>1000	>1000	977	899
		MONKEY	LIVER	189	247	440	241	>1000	>1000	>1000	>1000	628	731
TEST	4.40000 %	MOUSE	LIVER	18	21	13	11	19	16	32	29	142	159
	2.20000 %	MOUSE	LIVER	18	11	14	18	20	19	37	32	129	131
	1.10000 %	MOUSE	LIVER	22	19	19	21	15	14	37	49	157	148
	4.40000 %	RAT	LIVER	22	24	25	28	22	18	39	42	122	138
	2.20000 %	RAT	LIVER	21	25	22	28	22	22	28	39	144	152
	1.10000 %	RAT	LIVER	23	25	31	22	19	17	33	44	149	128
	4.40000 %	MONKEY	LIVER	16	20	17	14	13	10	41	44	202	221
	2.20000 %	MONKEY	LIVER	27	16	11	19	17	18	40	41	170	188
	1.10000 %	MONKEY	LIVER	15	11	18	15	22	11	28	41	213	227

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE
TA-1537 QM 20 UG/PLATE
TA-1538 NF 100 UG/PLATE
TA-98 NF 100 UG/PLATE
TA-100 MNNG 2 UG/PLATE

*** TA-1535 ANTH 100 UG/PLATE
TA-1537 AMQ 100 UG/PLATE
TA-1538 AAF 100 UG/PLATE
TA-98 AAF 100 UG/PLATE
TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS(UL) OR MICROGRAMS(UG) PER PLATE.

\- INDICATES NO DATA WAS TAKEN.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 12/06/77

NONACTIVATION COMPOUND 000000004

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		57.42	8.31	9.02	9.80	15.24	5.66	5.26	CONTROLS
NAP		229.21	100.40	105.08	55.35	72.08	48.23	50.97	
NA1		61.44	6.46	8.48	6.20	12.61	4.95	5.62	TEST DATA
NA2		62.16	6.27	6.56	13.86	9.34	3.32	4.17	
NA3		68.40	5.33	14.63	8.24	12.85	5.19	6.98	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 12/06/77

SPECIES ICRFLO/MOUSE COMPOUND 000000004

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	15.99	6.02	20.43	14.17	7.99	13.22	11.57	NEGATIVE CONTROLS
ACT	A-C	19.19	5.71	17.33	10.92	6.31	12.42	11.57	
ACT	ALI	53.46	4.64	17.88	9.80	14.48	12.10	9.96	
ACT	ALU	50.17	5.32	10.64	6.82	11.27	6.86	8.17	
ACT	PLI	65.79	81.16	118.33	85.23	122.21	71.83	70.48	POSITIVE CONTROLS
ACT	PLU	27.84	5.41	8.42	21.37	32.86	9.06	7.80	
ACT	LI1	52.12	6.64	9.48	7.24	11.06	9.31	8.98	TEST COMPOUND
ACT	LI2	19.29	5.20	7.06	6.07	10.26	8.32	10.33	
ACT	LI3	39.55	3.21	4.50	8.27	10.11	12.35	10.20	
ACT	LU1	39.17	3.84	4.99	8.47	2.88	10.94	10.29	
ACT	LU2	36.43	5.15	6.72	4.71	3.98	10.39	6.56	
ACT	LU3	45.11	4.50	6.39	9.47	15.06	11.06	12.00	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 12/06/77

SPECIES SPRDAW/RAT COMPOUND 000000004

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	23.84	2.68	20.32	4.37	26.72	8.48	7.51	NEGATIVE CONTROLS
ACT	A-C	27.40	3.60	17.83	4.20	10.46	7.79	7.58	
ACT	ALI	34.47	5.87	15.66	6.02	20.58	16.92	14.66	
ACT	ALU	40.61	3.57	15.48	6.45	16.55	12.92	15.05	
ACT	PLI	215.99	91.35	87.93	56.46	79.19	92.21	57.22	POSITIVE CONTROLS
ACT	PLU	20.79	3.89	6.53	7.37	19.26	8.07	7.50	
ACT	LI1	28.52	2.01	2.91	5.69	9.58	13.18	11.42	TEST COMPOUND
ACT	LI2	20.05	4.30	9.33	5.19	8.33	12.43	10.49	
ACT	LI3	20.88	4.03	6.51	3.59	22.16	9.77	8.95	
ACT	LU1	7.19	5.72	4.75	5.44	5.15	8.36	7.86	
ACT	LU2	21.94	4.37	5.46	5.09	10.78	13.17	11.74	
ACT	LU3	23.66	3.76	13.33	5.85	24.39	11.18	11.03	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 12/06/77

SPECIES RHESUS/MONKEY COMPOUND 000000004

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	28.29	4.82	17.11	4.33	7.89	7.93	8.48	NEGATIVE CONTROLS
ACT	A-C	25.91	3.16	14.44	2.79	6.98	11.80	12.23	
ACT	ALI	49.41	4.99	20.54	5.53	13.81	14.76	10.88	
ACT	ALU	31.95	4.91	21.96	5.13	18.92	13.70	11.48	
ACT	PLI	90.89	62.30	83.11	52.25	87.73	46.84	63.66	POSITIVE CONTROLS
ACT	PLU	26.91	3.97	8.42	6.51	14.84	12.76	11.84	
ACT	LI1	39.65	4.35	13.57	5.22	16.06	10.46	11.31	TEST COMPOUND
ACT	LI2	15.34	5.18	14.18	4.83	25.60	8.30	7.52	
ACT	LI3	12.97	3.97	16.81	4.23	7.06	9.08	8.82	
ACT	LU1	25.58	3.24	25.32	5.87	21.20	12.62	10.98	
ACT	LU2	33.73	2.64	30.96	5.28	13.06	9.29	10.79	
ACT	LU3	34.05	4.34	13.69	4.48	16.65	11.61	12.72	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) A+C = Negative Chemical Control for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU or A+T = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = x 10^6).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = 10^0). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.

DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (*Macaca mulatta*)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 71-12 Gum Tragacanth, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate test

The results of these tests were negative.

2. Nonactivation syspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

The test compound, FDA 71-12 Gum Tragacanth, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

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12/20/77

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Litton BIONETICS

VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagnes to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control values.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a buil-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or convertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is ALI or ALU > A-C > A+C.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a <u>set</u> of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727241 DETECTOR TA100 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1435	0824	57.42	0
	NAP		EMS 0.066%	1643	3766	229.21	0
000000004	NA1		0044-1 PCT.	0970	0596	61.44	0
000000004	NA2		0022-1 PCT.	1110	0690	62.16	1
000000004	NA3		0011-1 PCT.	0902	0617	68.40	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 722102 DETECTOR TA1535 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0602	0050	8.31	0
	NAP		EMS 0.2%	0506	0508	100.40	0
000000004	NA1		0044-1 PCT.	0650	0042	6.46	0
000000004	NA2		0022-1 PCT.	0765	0048	6.27	0
000000004	NA3		0011-1 PCT.	0676	0036	5.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727103 DETECTOR TA1537 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1331	0120	9.02	0
	NAP		QM 13 UG/ML	0413	0434	105.08	0
000000004	NA1		0044-1 PCT.	0908	0077	8.48	0
000000004	NA2		0022-1 PCT.	0610	0040	6.56	0
000000004	NA3		0011-1 PCT.	1435	0210	14.63	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 722103 DETECTOR TA1538 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	NAN		SOLVENT	0990	0097	9.80	0
	NAP		NF 667 UG/ML	0898	0497	55.35	0
000000004	NA1		0044-1 PCT.	0693	0043	6.20	0
000000004	NA2		0022-1 PCT.	0678	0094	13.86	0
000000004	NA3		0011-1 PCT.	0607	0050	8.24	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727102 DETECTOR TA98 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0899	0137	15.24	0
	NAP		NF 667 UG/ML	0684	0493	72.08	0
000000004	NA1		0044-1 PCT.	0555	0070	12.61	1
000000004	NA2		0022-1 PCT.	0685	0064	9.34	1
000000004	NA3		0011-1 PCT.	0599	0077	12.85	1

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 731501 DETECTOR 0000D4 SPECIES / DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0989	0056	0052	5.66	5.26	0
	NAP		EMS 1.0 %	0877	0423	0447	48.23	50.97	0
000000004	NA1		0005-0 PCT.	1211	0060	0068	4.95	5.62	0
000000004	NA2		0025-1 PCT.	1749	0058	0073	3.32	4.17	0
000000004	NA3		0125-2 PCT.	1232	0064	0086	5.19	6.98	0

REPORT EXP33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727204 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2145	0343	15.99	0
	A-C		SOLVENT	1024	0350	19.19	0
	ALI		TISSUE	1403	0750	53.46	0
	ALU		TISSUE	1431	0718	50.17	0
	ACP	LI	DMN 90 UM/ML	2216	1458	65.79	0
	ACP	LU	DMN 90 UM/ML	1225	0341	27.84	0
000000004	ACT	LI1	0044-1 PCT.	0495	0258	52.12	0
000000004	ACT	LI2	0022-1 PCT.	0622	0120	19.29	0
000000004	ACT	LI3	0011-1 PCT.	0794	0314	39.55	0
000000004	ACT	LU1	0044-1 PCT.	0822	0322	39.17	0
000000004	ACT	LU2	0022-1 PCT.	1290	0470	36.43	0
000000004	ACT	LU3	0011-1 PCT.	1053	0475	45.11	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 725202 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0515	0031	6.02	0
	A-C		SOLVENT	0508	0029	5.71	0
	ALI		TISSUE	0539	0025	4.64	0
	ALU		TISSUE	0601	0032	5.32	0
	ACP	LI	DMN 90 UM/ML	0499	0405	81.16	0
	ACP	LU	DMN 90 UM/ML	0592	0032	5.41	0
000000004	ACT	LI1	0044-1 PCT.	0467	0031	6.64	0
000000004	ACT	LI2	0022-1 PCT.	0558	0029	5.20	0
000000004	ACT	LI3	0011-1 PCT.	0591	0019	3.21	0
000000004	ACT	LU1	0044-1 PCT.	0521	0020	3.84	0
000000004	ACT	LU2	0022-1 PCT.	0582	0030	5.15	0
000000004	ACT	LU3	0011-1 PCT.	0555	0025	4.50	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727106 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0793	0162	20.43	0
	A-C		SOLVENT	1056	0183	17.33	0
	ALI		TISSUE	0800	0143	17.88	0
	ALU		TISSUE	1447	0154	10.64	0
	ACP	LI	AMQ 333 UG/ML	0180	0213	118.33	0
	ACP	LU	AMQ 333 UG/ML	1140	0096	8.42	0
000000004	ACT	LI1	0044-1 PCT.	0422	0040	9.48	0
000000004	ACT	LI2	0022-1 PCT.	0269	0019	7.06	0
000000004	ACT	LI3	0011-1 PCT.	0555	0025	4.50	0
000000004	ACT	LU1	0044-1 PCT.	0441	0022	4.99	0
000000004	ACT	LU2	0022-1 PCT.	0372	0025	6.72	0
000000004	ACT	LU3	0011-1 PCT.	0704	0045	6.39	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 722101 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0875	0124	14.17	0
	A-C		SOLVENT	0742	0081	10.92	0
	ALI		TISSUE	0714	0070	9.80	0
	ALU		TISSUE	0557	0038	6.82	0
	ACP	LI	ANTH 67 UG/ML	0623	0531	85.23	0
	ACP	LU	ANTH 67 UG/ML	0571	0122	21.37	0
000000004	ACT	LI1	0044-1 PCT.	0608	0044	7.24	0
000000004	ACT	LI2	0022-1 PCT.	0577	0035	6.07	0
000000004	ACT	LI3	0011-1 PCT.	0532	0044	8.27	0
000000004	ACT	LU1	0044-1 PCT.	0543	0046	8.47	0
000000004	ACT	LU2	0022-1 PCT.	0552	0026	4.71	0
000000004	ACT	LU3	0011-1 PCT.	0486	0046	9.47	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727101 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1614	0129	7.99	0
	A-C		SOLVENT	1695	0107	6.31	0
	ALI		TISSUE	0587	0085	14.48	0
	ALU		TISSUE	0896	0101	11.27	0
	ACP	LI	ANTH 67 UG/ML	1058	1293	122.21	0
	ACP	LU	ANTH 67 UG/ML	0423	0139	32.86	0
000000004	ACT	LI1	0044-1 PCT.	0398	0044	11.06	0
000000004	ACT	LI2	0022-1 PCT.	0380	0039	10.26	0
000000004	ACT	LI3	0011-1 PCT.	0475	0048	10.11	0
000000004	ACT	LU1	0044-1 PCT.	0868	0025	2.88	0
000000004	ACT	LU2	0022-1 PCT.	0954	0038	3.98	0
000000004	ACT	LU3	0011-1 PCT.	1049	0158	15.06	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 726402 DETECTOR 0000D4 SPECIES ICRFLO/MOUSE DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0968	0128	0112	13.22	11.57	0
	A-C		SOLVENT	0821	0102	0095	12.42	11.57	0
	ALI		TISSUE	1074	0130	0107	12.10	9.96	0
	ALU		TISSUE	1298	0089	0106	6.86	8.17	0
	ACP	LI	DMN 90 UM/ML	0891	0640	0628	71.83	70.48	0
	ACP	LU	DMN 90 UM/ML	0872	0079	0068	9.06	7.80	0
000000004	ACT	LI1	0005-0 PCT.	0913	0085	0082	9.31	8.98	0
000000004	ACT	LI2	0025-1 PCT.	0949	0079	0098	8.32	10.33	0
000000004	ACT	LI3	0125-2 PCT.	0980	0121	0100	12.35	10.20	0
000000004	ACT	LU1	0005-0 PCT.	1069	0117	0110	10.94	10.29	0
000000004	ACT	LU2	0025-1 PCT.	1174	0122	0077	10.39	6.56	0
000000004	ACT	LU3	0125-2 PCT.	1067	0118	0128	11.06	12.00	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727245 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	2731	0651	23.84	0
	A-C		SOLVENT	2788	0764	27.40	0
	ALI		TISSUE	1497	0516	34.47	0
	ALU		TISSUE	1407	0684	48.61	0
	ACP	LI	DMN 90 UM/ML	1301	2810	215.99	0
	ACP	LU	DMN 90 UM/ML	1703	0354	20.79	0
000000004	ACT	LI1	0044-1 PCT.	1017	0290	28.52	0
000000004	ACT	LI2	0022-1 PCT.	0728	0146	20.05	0
000000004	ACT	LI3	0011-1 PCT.	1695	0354	20.88	0
000000004	ACT	LU1	0044-1 PCT.	1474	0106	7.19	0
000000004	ACT	LU2	0022-1 PCT.	1568	0344	21.94	0
000000004	ACT	LU3	0011-1 PCT.	1399	0331	23.66	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 725203 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0634	0017	2.68	0
	A-C		SOLVENT	0528	0019	3.60	0
	ALI		TISSUE	0545	0032	5.87	0
	ALU		TISSUE	0532	0019	3.57	0
	ACP	LI	DMN 90 UM/ML	0520	0475	91.35	0
	ACP	LU	DMN 90 UM/ML	0592	0023	3.89	0
000000004	ACT	LI1	0044-1 PCT.	0944	0019	2.01	0
000000004	ACT	LI2	0022-1 PCT.	0674	0029	4.30	0
000000004	ACT	LI3	0011-1 PCT.	0621	0025	4.03	0
000000004	ACT	LU1	0044-1 PCT.	0612	0035	5.72	0
000000004	ACT	LU2	0022-1 PCT.	0595	0026	4.37	0
000000004	ACT	LU3	0011-1 PCT.	0772	0029	3.76	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727105 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0940	0191	20.32	0
	A-C		SOLVENT	1043	0186	17.83	0
	ALI		TISSUE	1175	0184	15.66	0
	ALU		TISSUE	1150	0178	15.48	0
	ACP	LI	AMQ 333 UG/ML	0381	0335	87.93	0
	ACP	LU	AMQ 333 UG/ML	0613	0040	6.53	0
000000004	ACT	LI1	0044-1 PCT.	0585	0017	2.91	0
000000004	ACT	LI2	0022-1 PCT.	0493	0046	9.33	0
000000004	ACT	LI3	0011-1 PCT.	1029	0067	6.51	0
000000004	ACT	LU1	0044-1 PCT.	0989	0047	4.75	0
000000004	ACT	LU2	0022-1 PCT.	0879	0048	5.46	0
000000004	ACT	LU3	0011-1 PCT.	1373	0183	13.33	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 725704 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0939	0041	4.37	0
	A-C		SOLVENT	0833	0035	4.20	0
	ALI		TISSUE	0931	0056	6.02	0
	ALU		TISSUE	1085	0070	6.45	0
	ACP	LI	ANTH 67 UG/ML	0797	0450	56.46	0
	ACP	LU	ANTH 67 UG/ML	0882	0065	7.37	0
000000004	ACT	LI1	0044-1 PCT.	0879	0050	5.69	0
000000004	ACT	LI2	0022-1 PCT.	0847	0044	5.19	0
000000004	ACT	LI3	0011-1 PCT.	0918	0033	3.59	0
000000004	ACT	LU1	0044-1 PCT.	0901	0049	5.44	0
000000004	ACT	LU2	0022-1 PCT.	0864	0044	5.09	0
000000004	ACT	LU3	0011-1 PCT.	0769	0045	5.85	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727104 DETECTOR TA98 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0685	0183	26.72	0
	A-C		SOLVENT	1185	0124	10.46	0
	ALI		TISSUE	0588	0121	20.58	0
	ALU		TISSUE	0568	0094	16.55	2
	ACP	LI	ANTH 67 UG/ML	0644	0510	79.19	0
	ACP	LU	ANTH 67 UG/ML	0841	0162	19.26	0
000000004	ACT	LI1	0044-1 PCT.	0574	0055	9.58	0
000000004	ACT	LI2	0022-1 PCT.	0804	0067	8.33	0
000000004	ACT	LI3	0011-1 PCT.	0537	0119	22.16	0
000000004	ACT	LU1	0044-1 PCT.	0680	0035	5.15	0
000000004	ACT	LU2	0022-1 PCT.	0640	0069	10.78	0
000000004	ACT	LU3	0011-1 PCT.	0783	0191	24.39	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 726403 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0932	0079	0070	8.48	7.51	0
	A-C		SOLVENT	0924	0072	0070	7.79	7.58	0
	ALI		TISSUE	0839	0142	0123	16.92	14.66	0
	ALU		TISSUE	0751	0097	0113	12.92	15.05	0
	ACP	LI	DMN 90 UM/ML	0526	0485	0301	92.21	57.22	0
	ACP	LU	DMN 90 UM/ML	0880	0071	0066	8.07	7.50	0
000000004	ACT	LI1	0005-0 PCT.	0683	0090	0078	13.18	11.42	0
000000004	ACT	LI2	0025-1 PCT.	0877	0109	0092	12.43	10.49	0
000000004	ACT	LI3	0125-2 PCT.	0983	0096	0088	9.77	8.95	0
000000004	ACT	LU1	0005-0 PCT.	0598	0050	0047	8.36	7.86	0
000000004	ACT	LU2	0025-1 PCT.	1116	0147	0131	13.17	11.74	0
000000004	ACT	LU3	0125-2 PCT.	1360	0152	0150	11.18	11.03	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727206 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	2513	0711	28.29	0
	A-C		SOLVENT	2752	0713	25.91	0
	ALI		TISSUE	2111	1043	49.41	0
	ALU		TISSUE	1709	0546	31.95	0
	ACP	LI	DMN 90 UM/ML	2645	2404	90.89	0
	ACP	LU	DMN 90 UM/ML	2155	0580	26.91	0
000000004	ACT	LI1	0044-1 PCT.	1082	0429	39.65	0
000000004	ACT	LI2	0022-1 PCT.	1695	0260	15.34	0
000000004	ACT	LI3	0011-1 PCT.	1388	0180	12.97	0
000000004	ACT	LU1	0044-1 PCT.	1075	0275	25.58	0
000000004	ACT	LU2	0022-1 PCT.	1530	0516	33.73	0
000000004	ACT	LU3	0011-1 PCT.	1624	0553	34.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 725701 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMN 90 UM/ML	0581	0028	4.82	0
	A-C		SOLVENT	0602	0019	3.16	0
	ALI		TISSUE	0681	0034	4.99	0
	ALU		TISSUE	0692	0034	4.91	0
	ACP	LI	DMN 90 UM/ML	0512	0319	62.30	0
	ACP	LU	DMN 90 UM/ML	0604	0024	3.97	0
000000004	ACT	LI1	0005-0 PCT.	0712	0031	4.35	0
000000004	ACT	LI2	0025-1 PCT.	0657	0034	5.18	0
000000004	ACT	LI3	0125-2 PCT.	0781	0031	3.97	0
000000004	ACT	LU1	0005-0 PCT.	0649	0021	3.24	0
000000004	ACT	LU2	0025-1 PCT.	0682	0018	2.64	0
000000004	ACT	LU3	0125-2 PCT.	0715	0031	4.34	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727202 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMQ 333 UG/ML	1046	0179	17.11	0
	A-C		SOLVENT	1219	0176	14.44	0
	ALI		TISSUE	0886	0182	20.54	0
	ALU		TISSUE	0920	0202	21.96	0
	ACP	LI	AMQ 333 UG/ML	1190	0989	83.11	0
	ACP	LU	AMQ 333 UG/ML	0962	0081	8.42	0
000000004	ACT	LI1	0044-1 PCT.	0420	0057	13.57	0
000000004	ACT	LI2	0022-1 PCT.	0543	0077	14.18	0
000000004	ACT	LI3	0011-1 PCT.	1202	0202	16.81	0
000000004	ACT	LU1	0044-1 PCT.	1189	0301	25.32	0
000000004	ACT	LU2	0022-1 PCT.	0969	0300	30.96	0
000000004	ACT	LU3	0011-1 PCT.	0869	0119	13.69	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 726501 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0578	0025	4.33	0
	A-C		SOLVENT	0682	0019	2.79	0
	ALI		TISSUE	0976	0054	5.53	0
	ALU		TISSUE	0994	0051	5.13	0
	ACP	LI	ANTH 67 UG/ML	0710	0371	52.25	0
	ACP	LU	ANTH 67 UG/ML	0753	0049	6.51	0
000000004	ACT	LI1	0044-1 PCT.	0728	0038	5.22	0
000000004	ACT	LI2	0022-1 PCT.	0746	0036	4.83	0
000000004	ACT	LI3	0011-1 PCT.	0710	0030	4.23	0
000000004	ACT	LU1	0044-1 PCT.	0715	0042	5.87	0
000000004	ACT	LU2	0022-1 PCT.	0852	0045	5.28	0
000000004	ACT	LU3	0011-1 PCT.	0892	0040	4.48	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 727203 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1406	0111	7.89	0
	A-C		SOLVENT	1491	0104	6.98	0
	ALI		TISSUE	0898	0124	13.81	0
	ALU		TISSUE	0502	0095	18.92	0
	ACP	LI	ANTH 67 UG/ML	0644	0565	87.73	0
	ACP	LU	ANTH 67 UG/ML	0829	0123	14.84	0
000000004	ACT	LI1	0044-1 PCT.	0467	0075	16.06	0
000000004	ACT	LI2	0022-1 PCT.	0918	0235	25.60	0
000000004	ACT	LI3	0011-1 PCT.	1020	0072	7.06	0
000000004	ACT	LU1	0044-1 PCT.	0665	0141	21.20	0
000000004	ACT	LU2	0022-1 PCT.	0605	0079	13.06	0
000000004	ACT	LU3	0011-1 PCT.	0961	0160	16.65	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 731901 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 12/06/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1085	0086	0092	7.93	8.48	0
	A-C		SOLVENT	1161	0137	0142	11.80	12.23	0
	ALI		TISSUE	0928	0137	0101	14.76	10.88	0
	ALU		TISSUE	1080	0148	0124	13.70	11.48	0
	ACP	LI	DMN 90 UM/ML	1076	0504	0685	46.84	63.66	0
	ACP	LU	DMN 90 UM/ML	0870	0111	0103	12.76	11.84	0
000000004	ACT	LI1	0005-0 PCT.	1176	0123	0133	10.46	11.31	0
000000004	ACT	LI2	0025-1 PCT.	1422	0118	0107	8.30	7.52	0
000000004	ACT	LI3	0125-2 PCT.	1168	0106	0103	9.08	8.82	0
000000004	ACT	LU1	0005-0 PCT.	1157	0146	0127	12.62	10.98	0
000000004	ACT	LU2	0025-1 PCT.	1270	0118	0137	9.29	10.79	0
000000004	ACT	LU3	0125-2 PCT.	1077	0125	0137	11.61	12.72	0